Effects of simulated acid rain on seedling emergence and growth of five broad-leaved species

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Abstract Seeds and seedlings of five broad-leaved species were separately exposed to simulated acid rain at pH values of 2.0, 3.5, 5.0, and 6.0, or to distilled water (the control). The results showed that seed germination was remarkably inhibited by pH 2.0 treatment for three species. Significant foliar damage, decline in chlorophyll contents, and retardation of the seedlings growth of all the species, were observed at pH 2.0 treatment. The pH 2.0 treatment seemed to be a threshold level for inhibition of seed germination and seedling growth for all the treated species, while seedling was stimulated at pH value between 3.5 and 5.0.
Key words: Simulated acid rain, Broad-leaved species, Seed germination, Seedling growth

Introduction

Acid rain emerged as an environmental issue of increasing concern in the late 1970s in China. The results of nationwide monitoring data gathered showed that widespread occurrence of acid rain was concentrated in southern part of the Yangtze River, especially in southwestern region, where precipitation in the late 1980s was found to have average pH values between 3.5 and 4.8 (Yang 1989; Xu 1990). Owing to the increasing seriousness of acid rain in southwestern China, a series of research programs have been set up by the National Environmental Protection Office since 1982 (Hang 1993).

Based on existing knowledge and theoretical postulations, the potential effects of acid rain on plants include as following: Injury to foliage; interference with normal metabolism; accelerated leaching of nutrients from plant foliage and soil; influences on seedling emergence and growth; alterations of symbiotic associations and host-parasite interactions; and increased susceptibility to some environmental stress factors (Wood & Bormann 1974; Tamm & Smith 1981; Pitelka & Raynal 1989). The experiment of simulated acid rain was usually considered and designed by many researchers as an effective way for determining the relative importance of these effects. And great progress has been made so far (Wood & Bormann 1976; Lee & Weber 1979; Mccoll & Robert 1983). In 1995, the effects of simulated acid rain on seed germination and seedling growth of five broad-leaved species were studied in the acid tolerance test in Fujian of China.

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Materials and methods

Germination test

Seeds of five broad-leaved species were placed in culture dishes with filter sheets to germinate in a culture container kept at a constant temperature of 25°C. Before experiment, the dishes were pasteurized at 100°C for one hour in oven, and seeds with 0.5% formalin solution for twenty minutes. The five species used in the test were *Cinnamomum camphora* L, *Ligustrum lucidum* Ait., *Castanopsis fissa* Rehd. et Wils., *Melia azedarach* L., and *Koelreuteria bipinnata* Franch. For each species, 25 dishes were randomly divided into five groups. The whole of 125 dishes was used, and each dish received 50 seeds. The filter sheets of dishes in each group were maintained in wet condition by spraying one simulated acid solution adjusted to one of the following pH values: 2.0, 3.5, 5.0, 6.0, and distilled water (the control). The simulated acid solution was prepared with solution of sulfuric and nitric acids in the ratio of 10 to 1 by chemical equivalents, with reference to the general anion composition of rainfall in South China. During the treatment, the numbers of germinated seeds were recorded each day.

Seedling spraying test

In December of 1994, 125 pots with each containing 7.5 kg of red earth from the Experimental Forest of Fujian Forestry College, Fujian, were randomly divid-

ed into five subsets, and placed in a greenhouse. In each subset of 25 pots, every 5 pots were planted with 50 seeds of one species (ten seeds in each of pots). The five species used were all the same as in germination test. The soils were irrigated with distilled water before treatment. About three weeks after seedling emergence were thinned to three seedlings in each of pots.

Beginning on May 10, 1995 and continuing for the next six months, every subset was subjected to one pH-level of simulated acid rain (prepared by the same method as in germination test). The acid rains and distilled water were applied once weeks during the first six weeks, afterwards twice a week, average 40 mm of solution per week.

On September 5, 1995, samples of fresh leaves were selected from each treatment. And the contents of chlorophyll a and b were measured by spectrophotometry. At the end of treatment (on November 5, 1995), the number of the total leaves and the leaves with visible necrosis for each seedling were recorded separately. The height and base diameter at root for each seedling were also measured. After that, the seedlings were harvested, and the leaves, shoots and roots were separated, oven-dried, and weighted.

Results and discussion

Effects on germination

The germination rates of different treatments of each species were given in Table 1. Based on *F*-test, significant treatment effects were observed for three species, in which germination rates were remarkably inhibited by acid solution at pH value of 2.0. *M. azedarach* and *L. lucidum* were not statistically significant. For all the species, however, continuous exposure to solution of pH 2.0 after the seeds germinating caused the primary shoots and roots of the tender seedlings to rot away gradually.

Table 1. Germination percentage of the seeds treated with simulated acid solution

Species	Treatment					Average value	Significance level*
	pH 2.0	pH 3.5	pH 5.0	pH 6.0	Distilled water		
Cinnamomum camphora	45.0	90.0	88.0	93.0	92.0	81.6	0.01
Ligustrum lucidum	21.0	19.0	24.0	26.0	24.0	22.8	NS
Castanopsis fissa	8.0	29.0	22.4	30.4	34.2	24.8	0.01
Melia azedarach	12.0	14.0	10.0	8.0	10.0	10.8	NS
Koelreuteria bipinnata	38.0	85.0	88.0	76.0	87.0	74.8	0.01

Note: * --determined from *F*-test. NS=not significant at $P<0.05$.

Effects on seedlings

Simulated acid rain produced severe damage to the leaves of all five species. The percentages of the damaged leaves at pH 2.0 were significantly higher than those at all other values of pH (Table 2). The initial symptom was the formation of marginal and interveinal necrotic spots with yellow-brown color. Eventually these necrotic zones extended through the leaves were visible on upper surfaces of leaves. Some damaged leaves curled as the necrotic areas dried. As shown in Table 2, overall leaf injury at pH 2.0 was the biggest for both *C. fissa and K. bipinnata.*

Table 2. Damage of simulated acid rain to seedling leaves, expressed by percent of numbers of the leaves damaged with visible necrosis

Species	Treatment				Distilled water	Average value	Significance level*
	pH 2.0	pH 3.5	pH 5.0	pH 6.0			
Cinnamomum camphora	51.7	14.2	6.3	6.4	1.8	16.1	0.01
Ligustrum lucidum	80.1	3.6	14.7	1.2	0	19.9	0.01
Castanopsis fissa	94.9	16.6	26.8	26.3	11.6	35.2	0.01
Melia azedarach	35.5	6.9	13.3	0	0	11.1	0.01
Koelreuteria bipinnata	99.4	18.9	18.6	12.3	13.8	32.6	0.01

Note: *-- determined from *F*-test.

The chlorophyll contents of the seedlings were decreased by simulated acid rain at lower pH levels (between 2.0 and 3.5), with the minimum chlorophyll contents at pH 2.0 for all the species, while the maximum chlorophyll contents occurred at pH levels between 5.0 and 6.0 (Table 3). However, the ratios of chlorophyll a and b were not significantly affected by the treatment for four species (except for *C. camphora*), the extent of chlorophyll a and b influenced was approximately the same.

Growth in height and diameter was significantly affected by the treatment for *C. fissa* and *M. azeda-*

rach, but adverse effects were confined to one measured parameter for the other three species (Table 4). As compared to the controls, at pH 3.5, height and diameter growth increased for *C. camphora, M. azedarach, K. bipinnata,* and only for height growth cf *L. lucidum*, but except for *C. fissa,* whose growth was the biggest when treated with distilled water.

Table 5 lists the average dry weight of the shoots leaves and roots of each species treated by simulated acid rain. All the species showed a significant inhibition of top and root growth at pH 2.0. The total of aboveground and underground dry weight was larger at pH levels between 3.5 and 5.0.

Table 3. Effects of simulated acid rain on chlorophyll contents of seedlings (mg/g)

Species	Treatment								Distilled water		Average value		Significance level*	
	pH 2.0		pH 3.5		pH 5.0		pH 6.0							
	chl. a+b	chl. a/b	chl. a+b	chl. a/b	chl. a+b	chl. a/b	chl. a+b	chl. a/b	chl. a+b	chl. a/b	chl. a+b	chl. a/b	chl. a+b	chl. a/b
C. camphora	0.729	1.21	0.986	1.13	1.134	1.14	1.311	1.23	1.292	1.25	1.090	1.19	0.01	0.05
L. lucidum	0.736	1.10	1.286	1.21	1.486	1.13	1.247	1.16	1.302	1.23	1.211	1.17	0.01	NS
C. fissa	0.675	1.16	0.814	1.10	0.942	1.15	1.003	1.17	0.904	1.22	0.868	1.16	0.05	NS
M. azedarach	0.767	1.13	1.386	1.17	1.462	1.19	1.046	1.13	1.238	1.16	1.180	1.16	0.01	NS
K. bipinnata	0.666	1.24	1.179	1.24	1.321	1.21	1.419	1.17	1.027	1.17	1.122	1.21	0.01	NS

Note: *-- determined from *F*-test, NS=not significant at $P<0.05$. chl. a+b and chl. a/b refer to the total contents of chlorophyll a and b, and the ratios of the two components, respectively.

Table 4. Effects of simulated acid rain on growth of height (*H*/ cm) and diameter (*D*/ mm) of the seedlings

Species		Treatment					Average value	Significance level*
		pH 2.0	pH 3.5	pH 5.0	pH 6.0	Distilled water		
Cinnamomum camphora	*H*	40.8	41.0	38.5	38.3	40.9	39.9	NS
	D	5.1	6.4	5.5	5.3	5.9	5.6	0.01
Ligustrum lucidum	*H*	25.0	31.9	31.4	28.2	30.4	29.4	NS
	D	2.5	3.3	3.2	3.5	3.5	3.2	0.01
Castanopsis fissa	*H*	15.5	21.2	13.4	14.2	23.1	17.5	0.01
	D	2.6	3.0	2.5	2.6	3.2	2.8	0.01
Melia azedarach	*H*	14.4	29.9	26.6	20.1	25.2	23.2	0.01
	D	3.4	5.3	4.9	5.0	4.3	4.6	0.01
Koelreuteria bipinnata	*H*	14.9	20.2	20.6	17.2	16.4	17.9	0.01
	D	6.2	6.5	6.4	6.2	5.7	6.2	NS

Note: *-- determined from *F*-test. NS=not significant at $P<0.05$.

Table 5. Average dry weight of seedlings treated with simulated acid rain (g)

Species		Treatment					Average value	Significance level*
		pH 2.0	pH 3.5	pH 5.0	pH 6.0	Distilled water		
Cinnamomum camphora	Shoot	0.594	2.294	2.314	2.049	1.647	1.780	0.01
	Leaf	1.465	4.020	3.639	3.205	3.535	3.173	0.01
	Root	0.763	3.189	2.565	2.713	2.325	2.311	0.01
Ligustrum lucidum	Shoot	0.255	0.423	0.944	0.633	0.729	0.597	0.01
	Leaf	0.235	0.740	1.817	1.162	1.241	1.039	0.01
	Root	0.163	0.669	0.581	0.490	0.536	0.488	0.01
Castanopsis fissa	Shoot	0.370	0.521	0.539	0.192	0.534	0.431	0.05
	Leaf	0.727	1.760	1.299	0.679	1.108	1.115	0.01
	Root	0.599	0.829	0.698	0.340	0.889	0.671	0.05
Melia azedarach	Shoot	0.132	1.616	1.045	0.680	0.781	0.851	0.01
	Leaf	0.114	1.997	1.062	0.793	0.705	0.934	0.01
	Root	0.125	1.979	2.180	2.965	1.631	1.776	0.01
Koelreuteria bipinnata	Shoot	0.613	1.366	1.709	1.502	1.113	1.261	0.01
	Leaf	1.220	2.935	3.247	3.134	2.640	2.635	0.01
	Root	1.721	2.633	2.613	3.015	2.851	2.567	0.01

Note: *-- determined from *F*-test.

The direction and magnitude of growth effects varied with species and with treatments, but generally pH 2.0 seemed to be a threshold level for inhibition of growth, while pH levels between 3.5 and 5.0 stimulated the growth.

Simulated acid rain at pH 2.0 in other experiments made damage to fine roots and increased leaching of soil cations, and the seedlings growth also decreased as a result of acceleration of respiratory rate (Shan and Hong 1989; Liao and Chen 1992). However, stimulation of seedling growth by simulated acid rain at pH values between 2.3 and 4.0 was estimated to be caused by NO_3^- fertilization, but accelerated productivity was supposed to be a short-term phenomenon due to declining supplies of available cations (Wood & Bormann 1976; Lee & Weber 1979). Under natural environment the positive or negative effects observed in artificial controlled conditions may be modified or a slow process, but changes in soil biological processes and chemical properties at acid rain have profound consequences on forest productivity (Tamm *et al.* 1976).

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